Replication Fork Breakage and Restart in Escherichia coli

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SUMMARY

In all organisms replication impairments are an important source of genome rearrangements, mainly because of the formation of double-stranded DNA (dsDNA) ends at inactivated replication forks. Three reactions for the formation of dsDNA ends at replication forks were originally described in *Escherichia coli*, and became seminal models for all organisms: the encounter of replication forks with pre-existing single-stranded DNA (ssDNA) interruptions, replication fork reversal, and head-to-tail collisions of successive replication rounds. Here, we first review the experimental evidence that now allows us to know when, where and how these three different reactions occur in *E. coli*. Then, we recall our recent studies showing that in wild-type *E. coli* spontaneous replication fork breakage occurs in 18% cells at each generation. We propose that it results from the replication of pre-existing nicks or gaps, since it does not involve replication fork reversal or head-to-tail fork collisions. In the *recB* mutant, deficient for double-strand break (DSB) repair, fork breakage triggers DSBs in the chromosome terminus during cell division, a reaction heritable for several generations. Finally, we recapitulate several observations suggesting that restart from intact inactivated replication forks and from recombination intermediates require different sets of enzymatic activities. The finding that 18% of cells suffer replication fork breakage suggests that DNA remains intact at most inactivated forks. Similarly, only 18% of cells need the helicase loader for replication restart, which leads us to speculate that the replicative helicase remains on DNA at intact inactivated replication forks and is reactivated by the replication restart proteins.

KEY WORDS: recombination, replication restart, PriA, RecA, RecBC, RuvAB, RecG, replication fork reversal, chromosome terminus, double-strand break.

INTRODUCTION

The two replication forks assembled at the replication origin of a bacterial circular chromosome progress in opposite directions until they meet in the terminus region, unless they are arrested by DNA damage or protein road-blocks. Obviously, proper chromosome replication is crucial because chromosomes can only be transmitted to progeny if they are fully replicated, but, in addition, replication fork arrest has dramatic consequences on genome stability. This idea emerged in studies of bacteria from the observation that mutations affecting DNA replication exhibited a hyper-recombination phenotype, and from the direct demonstration that blocked replication forks could be broken, and thus become entry points for DNA degradation or recombination, and, in turn, a source of DNA rearrangements (1-7). These concepts were soon extended to yeast and multi-cellular eukaryotes (8), for recent reviews see (9-12). The identification of the possible causes and consequences of accidental replication fork arrest and the description of replication restart pathways thus became the subjects of intense studies.

In this review we will first recall the molecular mechanism of homologous recombination at DNA double-strand (dsDNA) ends in *Escherichia coli*, and then present the three documented pathways of formation of dsDNA ends at inactivated replication forks. After that, we will discuss our recent study of the formation of spontaneous dsDNA ends in unchallenged *E. coli* cells, and finally describe how our results suggest important differences in replication restart reactions depending on whether the DNA at an arrested replication fork is broken or remains intact.

REPAIR OF DOUBLE-STRANDED DNA (dsDNA) ENDS IN *E. COLI*

The repair of dsDNA ends in *E. coli* starts by the action of RecBCD, a heterotrimeric complex with a helicase and a dsDNA exonuclease activity (reviewed in (13-15)). Its

exonuclease action (Exo V) is modified by the encounter of a specific site called Chi (5' GCTGGTGG 3'), which triggers loading of the recombinase RecA by RecBCD onto the 3'-ended DNA strand (Fig. 1A, repair of a dsDNA end). The RecA-ssDNA filament catalyses homology search and strand-exchange, which results in an X-like structure called a Holliday junction (HJ), adjacent to a displacement loop (D-loop) (Fig. 1A). HJs are specifically recognized and bound by RuvA and RuvB, which promote their migration, extending the heteroduplex sequence until the HJ-RuvAB complex is bound by the resolvase RuvC. RuvC resolves the HJ by cleavage of two opposite strands, and ligation produces two dsDNA recombinant molecules. Replication restart from recombination intermediates is essential for homologous recombination (16, 17). D-loops formed by strand invasion are, as replication forks, three-arm structures, with one ssDNA arm, two dsDNA arms and a 3' DNA end at the junction; therefore, they are targeted by PriA, the key enzyme for replication restart (18-21). This recognition of the strand invasion intermediate by PriA allows the reassembly of the replisome on the replication fork framework, formed by invasion, and triggers replication restart (Fig. 1A). PriA protein is a 3' to 5' helicase but its helicase activity is not required for restart (22). Note that DSBs are two-ended if they happen away from replication forks, while they have a one-ended configuration when they happen at replication forks. These two types of DSB can be differentiated experimentally (see for example Ref (23)). However, a DSB occurring close enough behind a fork is likely to be converted to a one-ended break by DNA degradation and will become indistinguishable from replication fork breakage.

FORMATION OF dsDNA ENDS AT REPLICATION FORKS IN *E. COLI*

In parallel to studies dedicated to replication restart, several investigations have aimed to understand how replication impairment can lead to the formation of dsDNA ends at forks. Although blocked forks might be inherently fragile owing to their ssDNA regions, only the

seqA mutant was proposed to suffer direct breakage of ssDNA at stalled replication forks (24, 25), and it turned out that most often arrested forks were not broken. Three main modes of dsDNA end formation at forks were reported: (i) encounter of a replication fork with a pre-existing single-stranded DNA interruption in a template strand (originally called "replication fork collapse" in a seminal review by A. Kuzminov, (26), Fig. 1B), (ii) replication fork reversal (27) sometimes also called replication fork regression (Fig. 1C), and (iii) encounter of a replication fork with a previously arrested fork, also called head-to-tail fork collisions, or fork rear-ending (28) (Fig. 1D).

Formation of a dsDNA end by the encounter of a replication fork with a single-stranded DNA interruption in a template strand.

An engineered ssDNA break is converted into a dsDNA end by the arrival of a replication fork ((29); Fig. 1B). In eukaryotic organisms site-specific or drug-induced ssDNA breaks were also shown to be converted into dsDNA breaks by the arrival of a replication fork (30, 31). The Kuzminov laboratory set out to identify mutations that increased the frequency of such chromosomal DSBs. Knowing that RecA is essential for the repair of dsDNA ends, they isolated mutations that are co-lethal with *recA* inactivation (32). Two mutations isolated perturbed the synthesis of deoxynucleotides (*tdk* and *rdgB*) and so directly implicated DNA replication. In these mutants, non-canonical deoxynucleoside triphosphates are not removed from the DNA precursor pools and are misincorporated into DNA during chromosome replication. They are then excised by a specific endonuclease, and this can lead to the formation of dsDNA ends in two ways: (i) formation of a two-ended DSB when two adjacent excision reactions occur, one on each DNA strand, and result in two nearly opposite single-stranded interruptions (Fig. 2), or (ii) formation of a single dsDNA end when a replication fork reaches a ssDNA break created by nucleotide excision in the template strand (Fig. 1B).

147 DSBs, formed in either way, are then repaired by the successive action of RecBCD, RecA,
148 RuvABC and PriA (Fig. 1A). However, the former happens at an undefined position behind
149 the replication fork while the latter only occurs at the replication fork when it reaches the
150 DNA interruption.

151 Several studies were carried out to differentiate between DNA breakage behind the
152 replication fork and at the replication fork. The *rdgB* mutant was shown to cause DSBs by
153 triggering the incorporation of xanthine and hypoxanthine into DNA, with their subsequent
154 excision by endo V, but the exact mode of DSB formation was not elucidated (33-35). The
155 *dut* mutant, which incorporates uracil into DNA, was also co-lethal with *recA* inactivation and
156 was shown by direct visualisation of chromosomes to suffer replication-dependent DSBs (36-
157 38). A detailed molecular analysis of chromosome breakage in a *dut recB* mutant suggested
158 that a combination of persistent ssDNA interruptions in the path of replication forks (Fig. 1B),
159 and clustered excision of misincorporated nucleotides on both strands (Fig. 2), are responsible
160 for DSB formation (38). The study was based on the idea that in a *recB* mutant replication-
161 dependent breakage generates exclusively origin-proximal and no origin-distal dsDNA ends
162 (the dsDNA end in Fig. 1B is linked to the origin, and thus called an origin-proximal end).
163 Origin-distal ends were observed, although at a lower frequency than origin-proximal ends.
164 Chromosome breakage in the *dut recB* mutant therefore results mainly from the encounter of
165 replication forks with ssDNA interruptions, and to some extent from clustered excision
166 behind replication forks (38).

167 Ligase mutants also require repair by recombination for viability and, since it was
168 thought that leading strand synthesis was continuous and ligases required to seal
169 discontinuous synthesis of the lagging strand, ssDNA breaks were originally believed to
170 accumulate only on the lagging strand. However, it was observed that chromosomes in a
171 ligase mutant accumulate nicks on both strands (39, 40). This finding suggested discontinuous

synthesis of both leading and lagging strands and raised the possibility of DSBs resulting from nicking across long-lived ssDNA breaks. Although DSBs in a weak ligase mutant could mainly result from replication forks reaching unsealed ssDNA breaks, DSBs in a strongly affected ligase mutant occurred most often behind replication forks, presumably resulting from nicks in close proximity on both strands, for example if a second nick in the opposite strand is preconditioned by, and targeted to, a first one that persisted because of the absence of ligase (41).

In conclusion, replication dependent fork breakage events occur when forks encounter ssDNA nicks or gaps on the template DNA and were observed readily in those cells that accumulate or fail to repair such nicks or gaps. However, in certain mutants, two-ended DSBs also happen behind replication forks, in addition to the one-ended breaks that happen at replication forks.

Replication fork reversal (RFR).

A screen for genes involved in replication fork breakage in the *E. coli* replication mutant *rep* led to the isolation of several mutations in the *ruvAB* operon, encoding proteins that act at Holliday junctions, and this observation gave rise to the replication fork reversal (RFR) model (27). According to this model, at certain blocked replication forks, such as in the *rep* mutant, the newly synthesized DNA ends anneal, forming a Holliday junction adjacent to a dsDNA end (Fig. 1C). In a cell proficient for the exonuclease V activity of RecBCD (Exo V) or homologous recombination, the dsDNA end is degraded or recombined. In cells deficient for both Exo V and homologous recombination (such as the *recBC* mutant), the HJ is resolved by RuvABC and the resulting linear chromosome arm is not repaired. Importantly, the RFR model proposed for the first time the formation of recombination substrates and the action of recombination proteins at blocked forks (Fig. 1C). The hallmarks of RFR are (i) a

requirement for RecBCD for viability (RecBCD can either degrade or recombine the dsDNA ends), (ii) no requirement for RecA provided that the exonuclease V action of RecBCD is active (requirement for either homologous recombination or linear DNA degradation), (iii) no measurable DNA degradation associated with replication inactivation in replication mutants that lack RecA (the degraded sequence is short), (iv) RuvABC-dependent chromosome breakage in the absence of RecBCD.

RFR was originally observed in *E. coli* in two helicase mutants, the *rep* null mutant that lacks an accessory replicative helicase, and in a *dnaBts* mutant where the main replicative helicase DnaB can be inactivated by a shift to a high temperature (27). In the *rep* mutant replication was thought to be arrested by protein road-blocks and thus, the role of Rep helicase was proposed to facilitate obstacle removal (27, 42). It was later shown that the primary role of Rep is to clear RNA polymerases from the path of replication forks and that Rep is present at forks by its interaction with the helicase DnaB (43-45). RFR also occurs in several other conditions of replication impairment: in replication mutants affected for different subunits of the holoenzyme polymerase III (46, 47), in the replication restart *priA* mutant (48), in mutants impaired for the biosynthesis of the nucleotide pool (49, 50), in UV-treated cells (51), in the presence of a topoisomerase inhibitor (52), in *Pseudomonas syringae* grown at low temperature (53), and in *Salmonella typhimurium* during nitrosative stress (54). In agreement with the original observation of RFR in an *E. coli rep* mutant, which lacks the main accessory helicase facilitating replication across DNA-bound proteins such as RNA polymerases (43, 55), RFR also occurred at an engineered strong replication-transcription collision site, where replication was arrested by an oppositely oriented, highly transcribed region (56). Finally, a helicase-driven RFR reaction was reported *in vivo* and *in vitro* for phage T4 (57). Note that reversed forks were proposed to form in eukaryotic cells and to be targeted by polymerases to allow lesion bypass (58), but the molecules observed in that work

were later shown to form *in vitro* during DNA extraction (59). Inter-conversion of replication and recombination intermediates in bacteria was also proposed theoretically (60).

In cells that undergo RFR, the enzyme that catalyses fork breakage was readily identified as RuvC. Two *E. coli* enzymes were shown to catalyse the conversion of replication forks into HJs *in vitro* and *in vivo*, both are homologous recombination enzymes: the recombinase RecA and the HJ branch migration enzyme RuvAB.

In *E. coli* replication fork reversal is catalysed by RecA or RuvAB.

RecA was the first enzyme shown to promote replication fork reversal: RuvABC-dependent breakage was abolished in the *dnaBts* mutant by the inactivation of RecA. It was proposed that RecA binding to the lagging strand template at a blocked fork could promote fork reversal by invasion of the homologous leading strand (Fig. 3A) (61). RecA-dependent fork reversal was also observed in UV-irradiated cells (51). This intra-molecular recombination reaction could be reconstituted on a DNA molecule mimicking a replication fork *in vitro* (62). A similar reaction was later shown to be mediated *in vivo* by the eukaryotic homologue of RecA, Rad51, following mild replication stress (63).

With the exception of *dnaBts*, RFR was independent of RecA in all bacterial replication mutants tested, suggesting the existence of other pathways. Genetic studies suggested that the helicase RecG might reverse forks in UV irradiated cells (64). This prompted the development of *in vitro* assays for RFR, based on the observation that *in vivo* HJs made by fork reversal are cleaved by the RuvABC HJ resolvase when the dsDNA end remains unprocessed ((27); Fig. 1C). Short DNA molecules that mimic replication fork structures were incubated with candidate enzymes, and the formation of a HJ was assayed by the addition of a resolvase (65, 66); later the formation of the fourth arm of the HJ was also monitored by restriction enzyme digestion (67). These experiments showed that *in vitro*

RecG could catalyse the conversion of a fork structure into a resolvase substrate. However, further *in vivo* studies did not confirm an active role of RecG in replication restart after UV irradiation (51, 68), and, to the contrary, the inactivation of *recG* promoted UV-induced replication (69, 70). Furthermore, testing RFR in different replication arrest conditions did not provide any evidence for a role of RecG *in vivo* ((56, 71); BM laboratory unpublished results). Accordingly, the actual *in vivo* RecG target was shown to be joint molecules made by homologous recombination. RecG works with RuvAB to prevent the unwinding of joint molecules (presumed to be RecA-mediated D-loops) by PriA helicase activity (72). *In vitro* and *in vivo,* RecG acts at D-loops in combination with the replication restart protein PriA: RecG orients the action of PriA, and, conversely, PriA binding prevents RFR by RecG ((23, 73-75); Fig. 4). Following the RecG studies, several eukaryotic helicases also have been shown to catalyse fork reversal *in vitro* (reviewed in (12, 75, 76)), but the lessons learnt from RecG clearly warn us that these structure-specific helicases do not necessarily reverse forks *in vivo*.

Replication fork reversal was shown to be catalysed by RuvAB in several *E. coli* replication mutants ((71); Fig. 3B). Indeed, in these replication mutants inactivating RuvAB prevented chromosome breakage by the alternative resolvase RusA, which indicated that RuvAB is necessary and sufficient for HJ formation at blocked forks (*ruvAB* inactivation abolished RFR, although all other helicases were expressed). *ruvA* and *ruvB* separation of function mutants were isolated, which were still fully functional for homologous recombination but unable to reverse forks (77-79). Biochemically, these RuvA mutant proteins were less efficient than wild-type RuvA for fork binding and for HJ branch migration in the presence of RuvB (77, 79). This result suggested that the conversion of a replication fork into a HJ (RFR) is a more demanding reaction than HJ branch migration. RFR is more difficult than HJ branch migration because the substrate of RFR has three DNA arms

including a ssDNA one; therefore there are less RuvA tetramer contacts with the DNA, and RFR starts with one RuvB hexamer bound to the three-strand junction. In contrast, the HJ has four dsDNA arms, therefore all RuvA monomers in the tetramers contact DNA and two RuvB hexamers bind to the structure (Fig. 3B). Accordingly, when a RuvAB-dependent RFR reaction was reconstituted *in vitro* on plasmid molecules, the branch migration reaction was so efficient that the HJs intermediate could not be trapped, and the short plasmid molecule carrying a blocked fork was entirely unwound by RuvAB (80).

It was also proposed that RFR could occur independently of any enzymatic activity, promoted by an excess of positive supercoiling at blocked forks (81). This idea was tested *in vivo* using a gyrase mutant (*gyrBts*) and a Topo IV mutant (*parEts*), in which positive supercoils created by transcription or replication are not efficiently removed at a high temperature, leading to replication fork blockage and lethality (82). Partial inactivation of gyrase or Topo IV caused replication fork arrest, as deduced from the need for the key replication restart protein PriA for viability. However, although arrested, forks blocked by positive supercoiling *in vivo* are not reversed, since RecBC was not essential for viability upon gyrase or Topo IV partial inactivation, and no increase in DSBs could be detected in the *gyrBts recB* mutant (83, 84). Furthermore, the *in vitro* experiments supporting supercoiling-driven RFR had used DNA incubation with a high concentration of intercalating agent, which is difficult to correlate with physiological conditions (81, 85, 86). Despite these reservations, the positive supercoiling-driven RFR reaction has been proposed on several occasions when the enzymes responsible for RFR could not be identified.

In conclusion, after the reconstitution of RuvAB-catalysed RFR *in vitro* (80) and our recent understanding of how PriA prevents RecG-catalysed RFR (23, 73-75), two modes of RFR reactions remain documented both *in vivo* and *in vitro* in *E. coli*: RecA-catalysed strand-exchange between leading- and lagging-strand ends (Fig. 3A), and RuvAB-catalysed

297 unwinding of a fork, converting it into a HJ (Fig. 3B). In addition, the UvsW helicase from
298 phage T4 was also shown to catalyze RFR both *in vivo* and *in vitro* (57).
299
300 **Formation of dsDNA ends by head-to-tail fork collision.**
301 Replication forks are naturally arrested in the chromosome terminus at specific *Ter*
302 sites by the encounter of *Ter*/Tus complexes (87). Use of ectopic *Ter* sites, introduced in the
303 chromosome to block replication progression, showed that replication forks arrested at an
304 ectopic *Ter*/Tus complex remained intact for one generation (28, 88). Chromosome labelling
305 experiments allowed us to conclude that dsDNA ends are formed when the following
306 replication round copied the blocked fork to the end, causing a head-to-tail fork collision
307 ((28), Fig. 1D) (also called fork rear-ending, (24)). Head to tail collisions were also proposed
308 to account for the observations in cells mutated for a subunit of the replicative DNA
309 polymerase, the holoenzyme polymerase III (89), in a *dnaAcos* mutant that suffers from
310 hyper-initiation at *oriC* (90) and in cells where hyper-initiation could be induced to a high
311 level at an engineered replication origin (91), and finally in a *seqA* mutant defective for sister-
312 chromatid cohesion ((25), but see also (24)). Interestingly, repair by RecBCD, RecA and
313 RuvABC-mediated recombination of the dsDNA ends made by re-replication of forks
314 blocked by a *Ter*/Tus complex was essential for viability (7, 28), suggesting that homologous
315 recombination allowed removal of Tus from the DNA. It turned out that replication forks
316 restarting from recombination intermediates differed from the originally arrested forks by
317 their accessibility to the fork-clearing helicase UvrD, which allowed restarting replication
318 forks to progress across the *Ter* site by displacing the DNA-bound Tus protein (92).
319
320 **THE GROWTH DEFECT OF *RECB* CELLS RESULTS FROM THE FORMATION**
321 **OF SIGMA-REPLICATING CHROMOSOMES.**

In addition to DSB repair, RecA is also essential for the repair by recombination of ssDNA gaps and for the SOS response, which is the induction by DNA damage of more than 40 proteins (reviewed in (93, 94)). Nevertheless, *recB* cells are less viable than *recA* cells, suggesting that the second function of RecBCD, dsDNA end degradation, is important for viability (95, 96). In mutants that undergo RFR, RecBCD degrades only a short tail and DNA degradation is too limited to be detected (27, 46). In contrast, extensive RecBCD-dependent chromosome degradation is observed in the *recA* single mutant, suggesting that dsDNA ends form in this mutant by a reaction other than RFR (97-99).

Fork breakage occurs in 18% of unchallenged wild-type cells per generation.

An important clue to the origin of the low viability of the *recBC* mutant came from the interesting observation of a deficit of DNA sequences in the chromosome terminus of the *recB* mutant (100, 101). We explored the reasons for this deficit by a combination of microscopy and marker frequency analyses, in minimal medium to prevent multi-fork DNA replication. We showed that terminus sequences were lost at the time of cell division, in one daughter cell only, in a division-dependent manner ((102); Fig. 5). Based on the observation that the phenomenon of terminus DNA loss was transmitted to progeny, we proposed and tested the model shown in Fig. 5 (103). In a first step, random replication fork breakage leads to the formation of a sigma-replicating chromosome, then the linear and circular parts segregate to the two cell halves, and finally they are separated by terminus DNA cleavage upon septum closure (Fig. 5). One of the daughter cells will never form a colony, as it contains a linear chromosome that is being degraded by nucleases, and the other one, which contains a circular chromosome with a linear tail, undergoes the same reaction again at each following generation (Fig. 5). Using fluorescence microscopy to measure cleavage of the chromosome terminus, we could show that the frequency of initial replication fork breakage

was 18% per cell per generation. As each cell harbours two replication forks, each individual replication fork has a 9% probability of being broken and not reaching the terminus. However, due to the heredity of the phenomenon, the percentage of dead cells in a *recB* exponential culture amounted to 32% (102).

Transmission of sigma-replicating chromosomes to progeny explains why most DSBs in a *recB* mutant occur in the terminus region, although the original DSBs occur at replication forks (two-ended chromosome DSBs, occurring elsewhere than at forks, would not lead to heritable terminus breakage, (103)). As one event of replication fork breakage triggered several rounds of terminus breakage, the model also explained why a high level of replication fork impairment was not observed in the *recBC* mutant (102, 104). However, while we now know that replication forks break at a frequency of 18% per cell per generation (9% per replication fork), we still do not know the molecular mechanism of fork breakage.

Spontaneous fork breakage may result from the encounter of a replication fork with a single-stranded DNA interruption in a template strand.

A putative role of RFR in the 18% spontaneous replication fork breakage was tested by microscopy, using the loss of labelled terminus DNA as an indication of chromosome terminus breakage and by comparing *recB* with *recB ruvAB* cells, and *recA recB* with *recA recB ruvAB* cells. The level of spontaneous replication fork breakage was identical in *ruv* mutants and Ruv^+ strains (21%), which strongly argues against replication fork reversal (103). Intriguingly, when linear DNA formation was measured by pulse-field gel electrophoresis (PFGE), the inactivation of *ruvAB* in a *recA recB* mutant decreased linear DNA formation two-fold, suggesting that fork breakage in this mutant occurred in part following RFR (47, 48). But in our study of terminus DNA loss, *ruvAB* inactivation only reduced the transmission of chromosome terminus breakage to the subsequent generations (from 84% in *recA recB*

cells to 60% in the *recA recB ruvAB* mutant), which remains unexplained. It did not affect spontaneous replication fork breakage (103), suggesting that RFR is not involved in fork breakage in wild-type *E. coli* growing in minimal medium; this presumably results from a high efficiency of the Rep helicase for the removal of protein road-blocks from the path of replication forks.

On the other hand, the fork rear-ending model implies dsDNA end formation by re-replication of blocked forks, thus one generation after replication fork blockage. Therefore, it is expected to cause a delay in cell division, while no cell-division delay or cell elongation was observed prior to terminus DNA loss (103). Thus, the most probable hypothesis remaining is that dsDNA end formation by replication of pre-existing ssDNA interruptions is the principal source of spontaneous double-strand breaks detected in a *recB* mutant. These events occur in 18% of cells per generation, therefore, on the 4.6 megabases (Mb) *E. coli* chromosome, ssDNA interruptions would be present with a frequency of 3.8×10^{-8} per base pair (1 per 26 Mb). Whether these ssDNA interruptions result from the repair of spontaneous DNA damage is presently unknown, but *recB* mutants are particularly sensitive to oxidative damage, and ssDNA breaks are putative intermediates in oxidative lesion repair ((105, 106), reviewed in (107)).

The repair of broken replication forks is predicted to lead to dimer chromosome formation when fork breakage occurs on the lagging strand, but not when it occurs on the leading strand (108). Dimers are resolved to monomers by *dif-dif* recombination catalysed by XerCD, and thus the frequency of formation of dimers was deduced from the frequency of exchanges between *dif* sequences (109, 110). Results in these two studies were slightly different but allow an estimation of 2% to 7.6% RecB-dependent dimer formation at each generation (by subtracting RecF-dependent dimers from the 10-16% total dimers measured in wild-type cells). These results suggest that fork breakage occurs on both strands, with

leading-strand breakage (caused by encounter of a ssDNA break on the previous lagging strand) occurring more often than lagging-strand breakage.

REPLICATION RESTART PATHWAYS

In *E. coli*, replication initiation at positions other than the replication origin can take place at inactivated intact replication forks (17), at recombination intermediates (16, 17) (Fig. 1A), and at R-loops in certain specific mutants, a phenomenon called constitutive stable DNA replication (cSDR, (111)). An R-loop is a three-arm structure that results from the stable pairing of a ssRNA molecule with one of the two dsDNA strands, and displacement of the homologous DNA strand. R-loops are recognized by PriA, as a 3' RNA end is present at the junction and, as at replication forks and D-loops, one arm is single-stranded and two arms are double-stranded. *oriC*-independent replication is affected by mutations in *priA, priB, priC, dnaT* and *dnaC* genes, alone or in combination (reviewed in (18-21)). DnaC is also required for replication initiation from the replication origin *oriC* and, accordingly, it catalyses the loading of DnaB at the chromosome origin *in vitro* (112, 113). In contrast, PriA, PriB, PriC and DnaT do not act at *oriC* but are specific for replication restart. PriA-dependent replication initiation was reconstituted *in vitro* on D-loops formed by RecA-mediated strand invasion (PriA substrates schematized at the bottom of Fig. 1A), and on naked DNA structures that mimic replication forks (intact replication forks schematized at the top of Fig. 1C). In both situations, this reaction required the sequential action of PriA, PriB, DnaT and DnaC: PriA targets D-loops or fork structures, promotes the binding of PriB and DnaT, which recruit the DnaC-DnaB complex for the loading of the replicative helicase DnaB on ssDNA (114-117). The similarity of the requirements for replication initiation from D-loops and forked structures *in vitro* led to the idea that *in vivo* restart from both inactivated intact forks and

421 recombination intermediates require the same set of proteins. As explained below, this may
422 not be the case.
423
424 **Replication restarts mainly from inactivated intact forks in wild-type untreated cells.**
425 A *priA* mutant does not propagate in rich medium and it grows slowly on minimum
426 medium, while a *priB priC* double mutant is dead (17, 118). By contrast, all recombination
427 mutants are viable on rich and on minimal media. The reduced plating efficiency of *recA* and
428 *recB* mutants is not as severe as that of a *priA* mutant grown on minimal medium (or the
429 complete loss of viability of the *priB priC* double mutant). Furthermore, the reduced plating
430 efficiency of these recombination mutants results in great part from their own defect in
431 homologous recombination, which triggers terminus DSBs (Fig. 5; (103)). Thus, the low
432 viability of replication restart mutants cannot be explained by a lack of replication initiation
433 from recombination intermediates and suggests that they fail to restart blocked forks that have
434 not recombined. Here, we will call such replication forks "inactivated intact forks", whose
435 DNA remains intact although replication elongation is arrested. The low viability of
436 replication restart mutants compared to recombination mutants thus suggests that these
437 inactivated intact forks are the main substrate for replication restart proteins in untreated wild-
438 type cells.
439 Epistatic interactions of mutations that inactivate the *priA*, *priB* or *priC* genes were
440 used to define replication restart pathways in otherwise wild-type cells (originally proposed in
441 (119), reviewed in (18, 20)) (Table 1, left column). The main replication restart pathway
442 requires PriA, DnaT, DnaC and either PriB or PriC (Table 1 Pathways 1A called PriA-PriB
443 and PriA-PriC below). The alternative PriC-DnaC pathway is poorly active, since it only
444 poorly supports viability of the *priA* mutant; other pathways are activated by *dnaC* mutations
445 (Table 1, left column). The PriA-PriC pathway (not reconstituted *in vitro*) is as efficient as the

PriA-PriB pathway in wild-type cells since the single *priB* mutant has no deleterious phenotype. However, the PriA-PriC pathway requires the helicase activity of PriA, specifically inactivated in a *priA300* mutant (22, 120). Indeed, the individual *priB*, *priC* and *priA300* single mutants are fully viable while the combination of *priB* inactivation with *priC* or *priA300* strongly affects viability, thereby suggesting that replication restart from inactivated intact forks is affected in these double mutants (118, 120).

Replication restart from inactivated intact forks and broken forks has different protein requirements.

The operation of these replication restart pathways can be deduced from the analysis of replication mutants in which PriA-mediated restart is essential for viability: (i) restart from inactivated intact replication forks is essential in a *gyrBts* mutant, which does not require homologous recombination for growth (83), (ii) restart from recombination intermediates is essential in a *dam* mutant, which requires homologous recombination for viability (121), (iii) restart after RFR is essential in the *rep* and *holD* mutants, which only require RecBC for viability (119, 122), and (iv) restart from R-loops is essential for the viability of a *dnaAts rnh* mutant which uses R-loops for replication initiation (111, 123, 124). PriB is essential for growth of *dam*, *rep*, *holD* and *dnaAts rnh* cells, while *gyrBts priB* cells are viable and only affected for growth in rich medium (83, 119, 122-124). Therefore, the PriA-PriB pathway is essential for replication restart from D-loops, reversed forks and R-loops, but not for replication restart from inactivated intact forks. Similarly, the *priA300* mutation that inactivates PriA helicase activity, barely affected the viability of *gyrBts* cells, whereas *rep priA300* and *holD priA300* showed a strongly reduced colony size, and *dnaA rnh priA300* cells were dead (83, 119, 122, 123). These observations suggest that replication restart from D-loops, reversed forks and R-loops require PriB and the helicase function of PriA (Table 1

Pathway 2A), while restart from inactivated intact forks does not (Table 1 Pathway 1A). Nevertheless, the requirement for PriB and PriA helicase activity for homologous recombination is not absolute, since *priB* and *priA300* mutants individually are not deficient for P1 transduction and not sensitive to low UV doses, in contrast with the *priB priA300* double mutant (120). There is either a qualitative difference between recombination after P1 transduction and in mutant strains that require recombination for viability (the PriA target is for some reason not exactly the same), or a quantitative difference (*priB* and *priA300* mutants can manage few recombination events, like during P1 transduction or at low UV doses, but cannot manage several recombination events per cell cycle like in *dam, rep* or *holD* mutants).

Studies of *dnaC* mutants further support the idea that restart from D-loops, reversed forks and R-loops do not require the same functions as restart from inactivated intact replication forks. Two *dnaCts* mutations inactivated replication initiation from *oriC* at high temperature while allowing most ongoing replication rounds to finish, which leads to their use in replication synchronisation experiments (*dnaC2ts* and *dnaC28ts*, (125)). This suggests that either a mutated DnaC protein with a residual replication restart activity was synthesized in these *dnaCts* mutants at a high temperature, or DnaC was not essential for replication restart from inactivated intact forks. To determine the proportion of cells that need intact DnaC protein for replication restart, these *dnaCts* mutations were used to synchronize replication in a cell population, and the proportion of chromosomes unable to complete a single round of replication was measured by flow cytometry (126). 18% of chromosomes remained partially replicated in this mutant, suggesting that replication was interrupted and did not restart in 18% cells at each generation in these *dnaCts* mutants. This is similar to the percentage of cells that suffer fork breakage ((103); Fig. 5), which suggests that DnaC may only be required in wild-type cells for replication initiation at the origin and for replication restart from recombination intermediates (Table 1, Pathway 2A) (126); note that the *dnaC2ts* mutant also

carries a *dnaT* mutation, but similar results were obtained with a *dnaC28ts* mutant excluding a role for the *dnaT* mutation). Accordingly, in the *rep* mutant where blocked forks are reversed, almost the whole cell population was unable to complete a single round of replication in a *dnaC2ts* mutant, indicating that reversed replication forks required wild-type DnaC for replication restart (126). In two other studies, replication restart occurred in the *dnaC2ts* mutant after induced replication arrest. Firstly, when replication fork arrest was increased by a gyrase ATPase inhibitor (which blocks replication without causing fork breakage or reversal, (83)), no chromosomes were fully replicated in a *priA* mutant but 60% of chromosomes were fully replicated in a *dnaC2ts* mutant (127). Secondly, in a study where replication forks were blocked by the encounter of a series of repressor-operator complexes, removal of these obstacles allowed 61% of replication forks to restart in a *dnaC2ts* mutant, versus 81% in wild-type and 17% only in a *dnaBts* mutant (128). Note that direct replication restart was quantified after a short time of replication arrest, while prolonged replication inhibition by these protein roadblocks led to RFR (128), as previously observed (56).

The behaviour of the *dnaC2ts* mutation points to a pivotal role of DnaC function in differentiating between inactivated intact forks, and D-loops, reversed forks or R-loops. This idea is supported by the properties of *dnaC* point mutants that activate PriA-independent pathways of replication restart or affect PriA-dependent replication restart (Table 1). *dnaC809* is a gain of function mutation that fully restores the viability of *priA* and *gyrBts priA* mutants, therefore allowing replication restart from inactivated intact forks in the absence of PriA (17, 83). In contrast, DnaC809 does not bypass the need for PriA in cells that undergo RFR or initiate replication from R-loops (119, 122, 123), although it restores P1 transduction in the *priA* mutant (17) (possibly because of a qualitative or a quantitative difference in the needs for replication restart between mutants that require it for viability and during P1 transduction, see above). Therefore, the *dnaC809* mutation allows replication restart in the absence of PriA

521 from inactivated intact forks, but not from D-loops, reversed forks, or R-loops, which
522 supports the idea that the role of DnaC is not the same in these different situations (Table 1
523 pathway 1C).

524 The *dnaC1331* mutation was isolated as affecting the replication of plasmids that
525 initiate replication from R-loops (Table 1 pathway 1D) (129). It does not affect the viability of
526 otherwise wild-type cells, which indicates that it does not affect restart from inactivated intact
527 forks. However, it is co-lethal with *rep* and *dam* mutations, indicating that, in addition to
528 restart from R-loops, it also affects restart from reversed forks and D-loops (124). This
529 phenotype is similar to that of the *priB* mutant described above and, accordingly, *dnaC1331* is
530 also strongly deleterious in combination with a *priA300* mutation (124). In conclusion,
531 *dnaC2ts*, *dnaC28ts*, *dnaC1331* and *dnaC809* are all dissociation of function mutations.
532 *dnaC2ts* and *dnaC28ts* inactivate at a high temperature replication initiation from the origin
533 *oriC* and replication restart from D-loops, while allowing replication restart from inactivated
534 intact forks. *dnaC1331* inactivates replication restart from D-loops, reversed forks and R-
535 loops, while allowing replication initiation at the origin and replication restart from
536 inactivated intact forks. *dnaC809* is a gain of function mutation that allows replication restart
537 in the absence of PriA from inactivated intact forks but not from D-loops, reversed forks or R-
538 loops. It should be noted that screening for suppressor mutations in a *priB priC* or *rep priB*
539 mutant yielded, as expected, *dnaC* alleles that did not show a dissociation of function
540 phenotype, but similarly bypassed replication restart proteins at inactivated intact forks and at
541 D-loops, reversed forks or R-loops (*dnaC824* and *dnaC809 820,* Table 1 Pathway 1E and 1F)
542 (reviewed in (18, 20)).

543 We can speculate that the PriA PriC pathway is active only at intact inactivated forks
544 (Table 1, Pathways 1A) and may not require an intact DnaC protein because it reactivates a
545 DnaB helicase left on DNA after replication arrest. DnaB forms a hexameric complex that

546 encircles the lagging-strand template and, to date, no mechanism of removal of the DnaB
547 helicase from the DNA has been described. Furthermore, *in vitro* DnaB progresses very
548 slowly on DNA in the absence of the holoenzyme polymerase III (HE Pol III) (130), and a
549 slight progression of DnaB at inactivated intact forks would render single-stranded both DNA
550 template strands creating the preferred substrate for PriC, or might allow a progression of the
551 lagging-strand end beyond the leading-strand end, accounting for the need for PriA helicase
552 activity for replication restart by the PriA-PriC pathway (116, 131). If DnaB remains bound to
553 inactivated intact forks, it may allow PriA-dependent replication restart in a *dnaC2ts* mutant
554 (Table 1, Pathway 1A), and PriA- or PriB-independent restart in *dnaC809* and *dnaC1331*
555 mutants, respectively (Table 1, Pathways 1C and 1D). *In vitro* a pre-loaded DnaB helicase is
556 sufficient for replication initiation in the presence of primase and HE Pol III (132, 133). We
557 hypothesize that *in vivo,* this reaction would need some replication restart proteins.
558
559 **Three other observations support the idea that different pathways may restart**
560 **replication at inactivated intact fork and at D-loops, reversed forks or R-loops.**
561 Firstly, in a microscopy study of the *priA* mutant two types of cells could be observed:
562 84% cells looked like wild-type, while 16% were elongated with a poorly partitioned
563 chromosome (134, 135). This phenotype was eliminated by mutating either *recA* or *recB,*
564 strongly suggesting that the cells with poorly partitioned chromosomes might have suffered
565 replication fork breakage (in a *priA* mutant, homologous recombination will be blocked after
566 the formation of a D-loop, owing to the lack of replication restart, Fig. 1A). The observation
567 of 84% normal cells was surprising considering the low viability of the *priA* mutant. This
568 experiment suggests that blocking replication restart from RecA-made D-loops has severe
569 consequences on chromosome partitioning, in contrast with blocking replication restart from
570 inactivated intact forks.

Secondly, in contrast with the major fork-clearing helicase Rep, which interacts with DnaB and presumably acts directly at intact inactivated forks (45), two observations suggest that the alternative fork-clearing helicase UvrD can only access blocked replication forks after recombination or reversal. UvrD is present and active in the *rep* mutant, yet at the same time fork reversal occurs in the *rep* mutant, showing that UvrD can only replace Rep after RFR has taken place (27, 43). UvrD is also essential for replication restart at forks blocked by an etopic replication terminator, but again it does not remove the Tus protein directly from blocked forks, since homologous recombination is required for its action (92). Further studies showed that yet another accessory helicase, called DinG, acts with UvrD at restarting replication forks blocked by RNA polymerases in a *rep* mutant (43). This raised the proposal that only replication forks reassembled after reversal or homologous recombination may be specifically accessible to certain fork-clearing proteins.

Thirdly, yet another helicase, RecG, acts at replication forks; moreover, suppressors of the *recG* mutant defects mapped in *priA* (136). However, the nature of the interplay between RecG and PriA remained unclear for a long time (137). Recently, marker frequency analysis by genomic sequencing and RecA binding by ChIP-seq were carried out in a *recG* mutant and revealed DNA synthesis proceeding in the opposite direction to that predicted for repair of a DSB (Fig. 4). This reaction called “reverse restart” was observed specifically in the absence of RecG, at a DSB generated at the site of a long DNA palindrome cleaved by SbcCD, and between dsDNA ends located at the termination sites *TerA* and *TerB* in the terminus of the chromosome (23). Based on the biochemical demonstration of correct loading of PriA at a replication fork substrate in the presence of RecG and prevention of RecG-mediated replication fork reversal by PriA *in vitro* (73, 74), a specific role for RecG in reverse restart was proposed (Fig. 4) (23, 75). In the absence of RecG, PriA could be loaded incorrectly at a replication fork, or a D-loop generated by recombination at the site of a DSB and at similar

structures generated at *Ter* sites in the chromosome terminus (57). This reverse restart reaction explains the over-replication previously observed in the absence of RecG following UV irradiation and in the chromosome terminus (70, 100, 101, 138). In agreement with the reverse restart model and with the idea that PriB and the helicase function of PriA are required for *de novo* DnaB loading (see above, Table 1), these two functions were required for over-replication in the chromosome terminus of the *recG* mutant (100).

Several observations suggest that, by driving progression of replication forks from terminus to origin, reverse restart should cause a growth defect (56, 139). However, in the *recG* mutant the proportion of cells with a growth defect is close to the proportion of cells that suffer fork breakage, around 15% (140, 141). This is in agreement with the idea that RecG acts during DSB repair (23), but not at inactivated intact forks. We propose that RecG is not needed for a proper binding of PriA at intact inactivated replication forks because DnaB is already present and in the correct position for restart.

CONCLUSIONS AND PERSPECTIVES

We have demonstrated recently that replication fork breakage is, as suspected, the major source of spontaneous DSBs in *E. coli*, and that preventing broken fork repair triggers heritable cell division-dependent DSBs in the chromosome terminus (103). The molecular mechanism of DSB formation in the terminus during cell division remains to be identified. Our results lead us to propose that replication fork breakage results mainly from the encounter of nicks or gaps in the template strands. These might form during the repair of oxidative DNA damage, but this hypothesis needs further investigation. The observation that a similar proportion of *E. coli* cells undergo replication fork breakage (103) and require helicase reloading to complete replication (126) leads us to speculate that the loading of a new helicase is only needed after replication fork breakage. Consequently, we propose that when the DNA

at inactivated replication forks is intact, the replication restart proteins may reactivate the helicase left on DNA after replication arrest, by promoting the binding of a new replisome to the abandoned helicase. This new proposal will of course need to be explored in the future to be validated.

ACKNOWLEDGMENTS.

Work in BM laboratory is supported by the Agence Nationale de la Recherche, ANR grant #11 BSVS5 006 01. Work in DL laboratory is supported by grant MR/M019160/1 from the Medical Research Council (UK). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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994 **FIGURE LEGENDS**

995 Figure 1. A: Repair of a broken replication fork by RecBCD, RecA, Ruv, PriA. RecBCD
996 binds to DNA double-strand ends, degrades both strands until it encounter a Chi site, at which
997 it loads RecA onto the 3' DNA end. The RecA-ssDNA filament invades a homologous
998 region, promotes strand exchange, resulting in a Holliday junction (HJ), indicated by the blue
999 and red crossing lines, and an adjacent displacement loop, also called D-loop, schematized by
1000 the displacement of one of the red lines by the blue line end. RuvAB binding to the HJ drives
1001 branch-migration. After RuvC binding, the RuvABC complex catalyzes resolution of the HJ,
1002 resulting in a recombinant molecule. PriA restarts replication from the D-loop. B: formation
1003 of a "broken fork" by the encounter of a pre-existing nick. An ssDNA break is drawn here on
1004 the lagging-strand template, but the same reaction occurs with a ssDNA break on the leading-
1005 strand template. DSB repair is as in A and reconstitutes a replication fork. C: replication fork
1006 reversal. In the first step (a), the replication fork is arrested, and the leading and lagging
1007 strand ends of the newly synthesized strands anneal. The resulting structure is called a
1008 reversed fork, it has a four-arm structure akin to a HJ; two alternative representations of this
1009 structure are shown, called open X and parallel stacked X. RecBC acts on the dsDNA end (as
1010 shown in A) and is essential for resetting of the fork, either by RecA-dependent homologous
1011 recombination (b-c) or by DNA degradation (b-d). Either pathway creates a substrate for
1012 replication restart proteins (PriA and its partners), since homologous recombination leads to a
1013 D-loop as shown in A, and DNA degradation restores a fork structure. In the absence of
1014 RecBCD, resolution of the HJ causes chromosome linearization (not shown). D: dsDNA ends
1015 formed by head-to-tail collision of replication forks. The dsDNA ends formed by re-
1016 replication are recombined as in A. This reaction occurs at forks blocked at an ectopic *Ter*
1017 site, where it requires UvrD to dislodge the Tus protein (see text). In A the blue and red
1018 continuous double lines represent two homologous DNA molecules. In B-C the continuous

lines represent the parental chromosome and the dashed lines represent the newly-synthesized strands. In D the dashed lines represent the DNA synthesized in a second replication round. Arrowheads show DNA 3' ends. Incised grey circles, RecBCD; small yellow circles, RecA; green circles, RuvAB.

Figure 2. Model for two-ended break repair following excision of two closely-spaced lesions on opposite strands of the DNA. When two lesions (denoted by X) are closely spaced on opposite strands of the DNA, their nucleolytic excision can lead to a two-ended double-strand break behind the replication fork. One of the ends, closest to the origin of replication, is denoted the Origin Proximal End and the other end, closest to the terminus of replication, is denoted the Origin Distal End. Each end is processed by RecBCD enzyme, which loads RecA protein. RecA protein catalyzes strand invasion to form two D-loops, and two HJ that are resolved by RuvABC. This converts the joint molecules to converging replication forks, which assemble new replisomes through PriA-dependent restart. The blue and red continuous double lines represent two homologous DNA molecules. Arrowheads show DNA 3' ends.

Figure 3. A: Model of RFR by RecA. RecA binding to the ssDNA region on the lagging-strand template of a blocked fork can promote the invasion of the homologous sequence on the leading strand. This reaction produces a reversed fork. B: Model of RFR by RuvAB. The RuvAB complex formed on a replication fork can only contain one RuvB hexamer. Branch migration promoted by this RuvB hexamer extrudes a dsDNA end on which a second RuvB hexamer can bind, resulting in a RuvAB-HJ complex similar to the one formed during homologous recombination. RuvC resolves the HJ, which in the case of a reversed fork results in fork breakage. Continuous lines, parental DNA strands; dashed lines, newly synthesized DNA strands; small yellow circles, RecA; orange trefoil RuvA tetramer; green circles, RuvB.

Arrowheads show DNA 3' ends. The small black arrows indicate the direction of strand displacement by the RuvAB complex.

Figure 4: Model of RecG and PriA concerted action at forks. It has been shown that RecG remodels replication forks *in vitro*: (Ai) when RecG is alone, this remodeling causes RFR. (Aii) when both RecG and PriA are present, PriA binds to the 3' end at the fork, preventing its unwinding by RecG; this reaction is likely to also take place *in vivo*, since no genetic evidence for RecG-dependent RFR could be obtained. (Bi) it has been proposed that *in vivo*, in addition to preventing RFR, the binding of PriA in the presence of RecG leads to the correct loading of DnaB to the lagging strand template. Because the PriA helicase domains, represented as a small orange star in the B panels, bind to the lagging-strand template, replication restarts in the initial direction. (Bii) in the absence of RecG, the PriA helicase domains bind to the newly-synthesized lagging-strand and, consequently, PriA can load DnaB incorrectly to this strand. This results in reverse restart, the assembly of a replication fork proceeding in the wrong direction. Large blue lines, template strands; red lines, newly synthesized strands; small blue lines in B (ii), strand synthesized by reverse restart; green crescent, RecG; purple star, PriA; small orange star in the B panels, PriA helicase domain; blue ring, DnaB replicative helicase; arrowheads show DNA 3' ends.

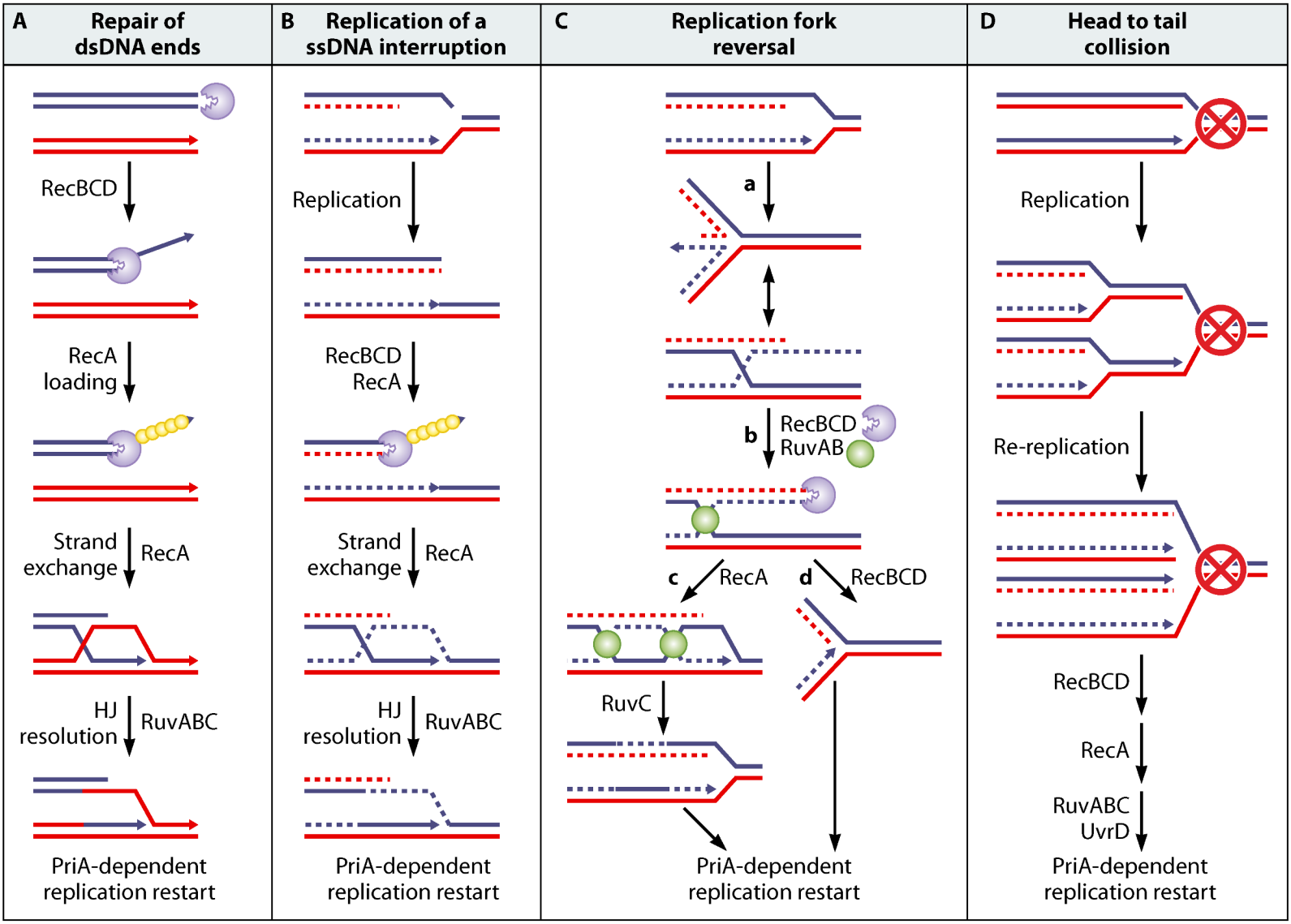
Figure 5: Model for terminus DNA loss in the *recB* mutant. In a first step a replication fork broken at a random position remains unrepaired in a *recB* mutant, resulting in the inability to complete one chromosome. The two daughter chromosomes, one truncated and one entire, are linked by the intact replication fork and segregate to the two cell halves. In a second step the terminus region of the truncated chromosome becomes trapped in the septum and is broken at the *dif* site during cell division. A non-viable cell with a linear chromosome and a viable cell

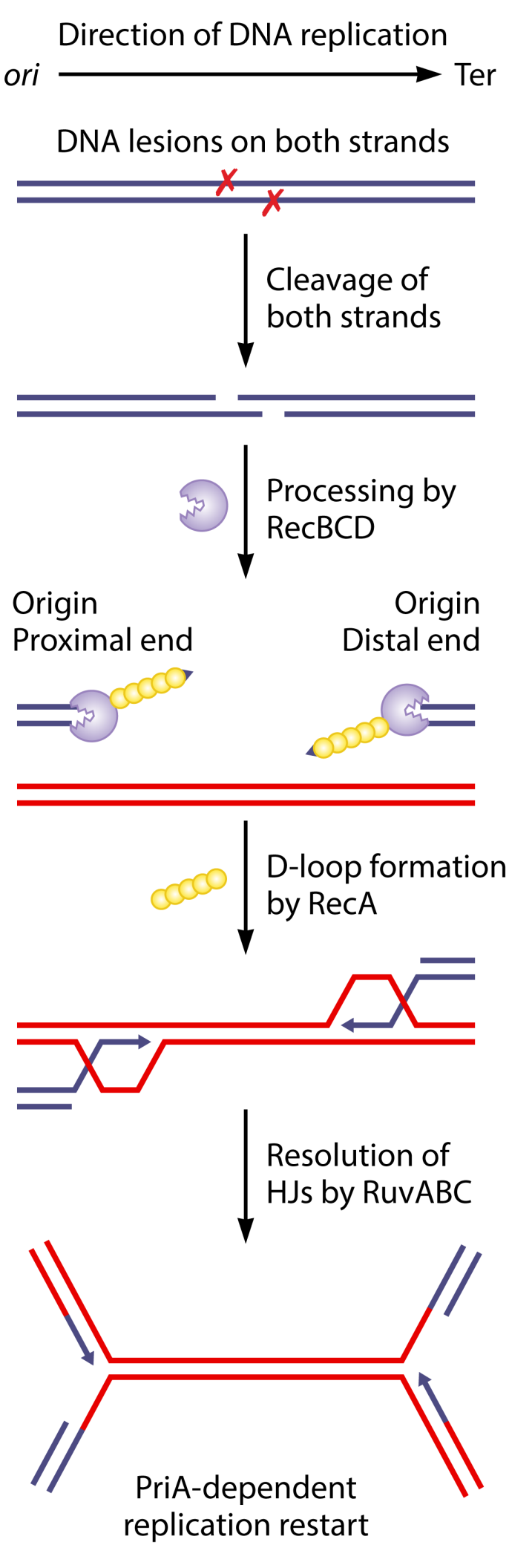
with a sigma-replicating chromosome are generated. In a third step the intact replication fork on the sigma-replicating chromosome meets a fork coming from the origin, which extends the small tail by the entire chromosome arm. This leads to the same substrate as originally generated by the fork breakage event, except that the end of the chromosome arm results from terminus breakage. Breakage of the new terminus DNA will occur again at the next cell division, generating a non-viable cell with a full linear chromosome and a viable cell with a sigma-replicating chromosome, and the same reaction can take place for several generations. The reader is referred to (103) for a more detailed depiction of these events. Light blue lines, bacteria; dark blue lines, DNA; large red arrows, DNA breaks. The positions of the replication origin *oriC* and of the last segregated sequence in the terminus, the *dif* site opposite to *oriC*, are indicated.

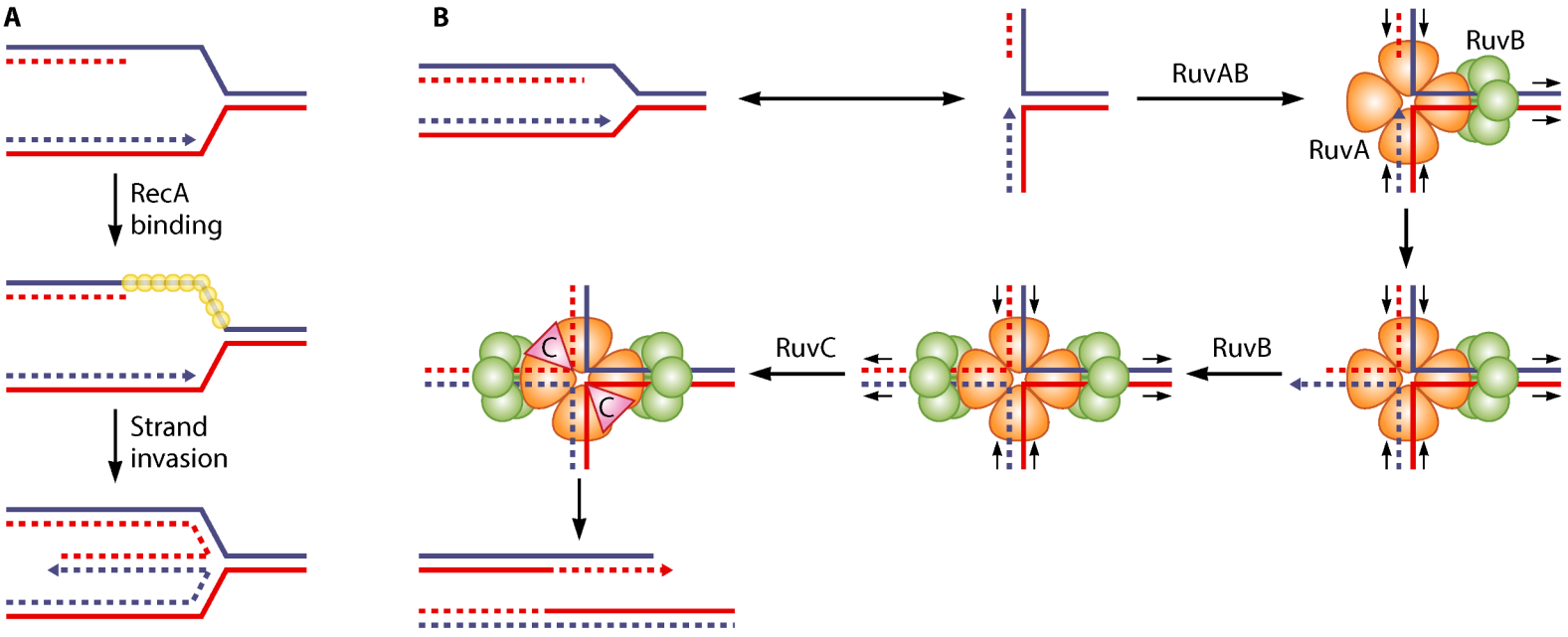
Table 1: Model of replication restart pathways.

1) Restart from inactivated intact replication forks (essential for viability)	**2) Restart from D-loops, reversed replication forks and R-loops.**
A - wild-type pathways: PriA – PriB or PriC – DnaT – (DnaC)	A - wild-type pathway: PriA – PriB – DnaT – DnaC
B - minor pathway: PriC – DnaC	inactive
C - suppressor pathways C: PriB or PriC – DnaC809	inactive
D – mutant *dnaC1331* pathway: PriA - PriC – (DnaT) - DnaC1331	inactive
E - suppressor pathways E: (PriA) - DnaC824	E – suppressor pathway E: (PriA) – DnaC824
F – suppressor pathway F: DnaC809 820	F – suppressor pathway F: (PriB) -DnaC809 820

Replication restart pathways were originally defined in Ref (119) and later modified in Ref (18). We propose here that different pathways operate depending on whether replication restarts from inactivated intact forks (left column) or D-loops, reversed forks or R-loops (right column. A - In wild-type cells replication can restart from inactivated intact forks via either of two pathways: PriA-PriB-DnaT or PriA-PriC-DnaT. DnaC is not needed for replication restarts from inactivated intact forks in a *dnaC2ts* and a *dnaC28ts* mutant at restrictive temperature. In contrast, PriB and DnaC are both essential for restart from D-loops, reversed forks and R-loops (together with the helicase function of PriA, not shown here). B - In the absence of PriA a minor PriC-DnaC pathway allows restart from inactivated intact forks, but not from D-loops, reversed forks and R-loops. C - In the *dnaC809* mutant this pathway is strongly activated; it can use either PriB or PriC but does not need PriA and DnaT. D - The *dnaC1331* mutation blocks the PriA-PriB replication restart pathway, leaving only the PriA-PriC pathway at inactivated intact forks (DnaT was not tested). E - The *dnaC824* mutation bypasses the need for PriB and PriC and only partially bypasses PriA at inactivated intact replication forks (DnaT was not tested). It bypasses PriB and PriC at D-loops and reversed forks (PriA and R-loops were not tested). F - The *dnaC809 820* mutation bypasses all other replication restart proteins at inactivated intact forks as at D-loops and reversed forks, but needs PriB for replication initiation at R-loops.


A
Repair of dsDNA ends
RecBCD
RecA loading
Strand exchange
RecA
HJ resolution
RuvABC
PriA-dependent replication restart
B
Replication of a ssDNA interruption
Replication
RecBCD RecA
Strand exchange
RecA
HJ resolution
RuvABC
PriA-dependent replication restart
C
Replication fork reversal
a
b
RecBCD RuvAB
c
RecA
d
RecBCD
RuvC
PriA-dependent replication restart
D
Head to tail collision
Replication
Re-replication
RecBCD
RecA
RuvABC UvrD
PriA-dependent replication restart


Direction of DNA replication
ori
Ter
DNA lesions on both strands
Cleavage of
both strands
Processing by
RecBCD
Origin
Proximal end
Origin
Distal end
D-loop formation
by RecA
Resolution of
HJs by RuvABC
PriA-dependent
replication restart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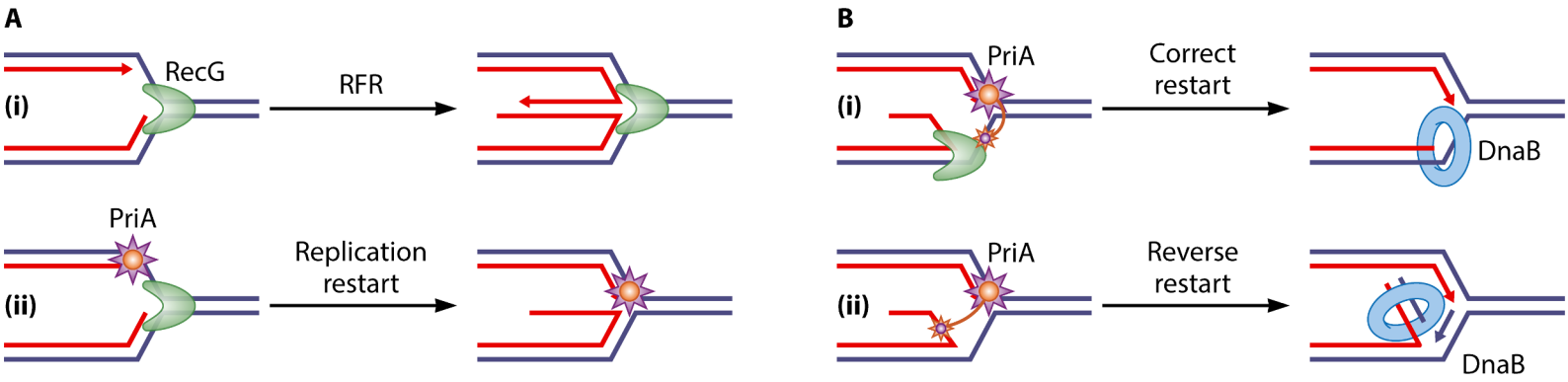
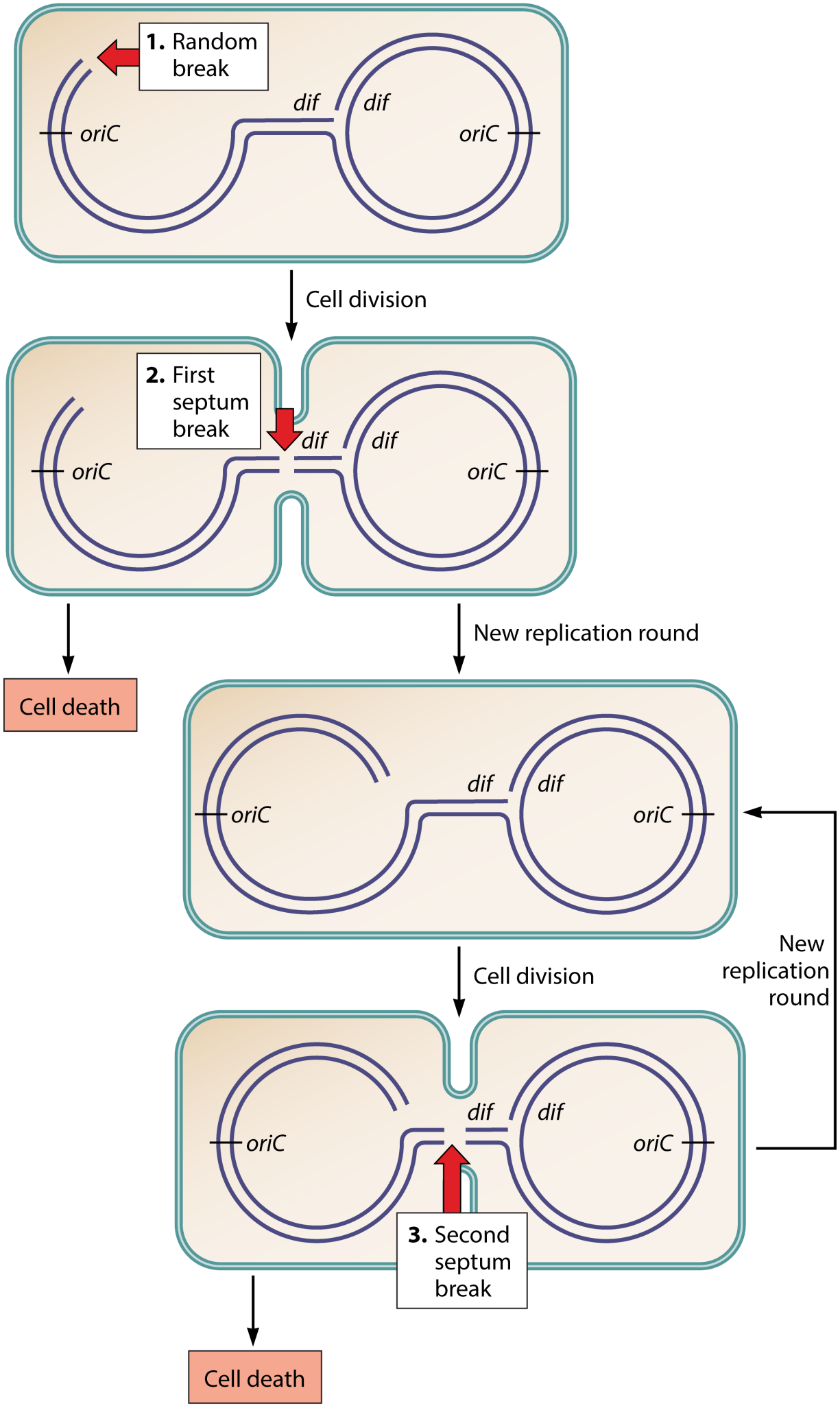
RecA
binding
Strand
invasion
B
RuvAB
RuvB
RuvA
RuvB
RuvC
C
C


A
(i)
RecG
RFR
(ii)
PriA
Replication
restart
B
(i)
PriA
Correct
restart
DnaB
(ii)
PriA
Reverse
restart
DnaB


1. Random break
dif
dif
oriC
oriC
Cell division
2. First septum break
dif
dif
oriC
oriC
Cell death
New replication round
dif
dif
oriC
oriC
Cell division
New replication round
dif
dif
oriC
oriC
3. Second septum break
Cell death